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The DNA damage-signaling pathway has been implicated in the development of prostate cancer. Germline mutations in several genes (*BRCA1, BRCA2, and CHEK2*) whose products are involved in this pathway have been associated with increased risk for this disease. To identify additional genes in this pathway that might confer susceptibility to prostate cancer, we isolated a p73 up-regulated gene (p73R1) and screened this gene for mutations in prostate cancer. Two germline truncating mutations were identified. Genotyping of 403 men with sporadic prostate cancer for the two mutations showed a frequency of 3.2% (13/403) in contrast to 0.6% (2/327) in 327 population-based controls (Fisher's exact test, $P = 0.016$), with an odds ratio of 5.4 (95% confidence interval 1.2 – 24.2). Analyses of 994 affected men from 444 familial prostate cancer families showed a relatively lower frequency of 1.6% but no mutations were found in 100 unaffected men from these families, indicating a similar trend observed for other comparisons. Overall, our data suggest that germline *p73R1* truncating mutations may predispose men to prostate cancer and further supports the concept that the mutant alleles in the DNA damage-response genes play an important role in the development of sporadic prostate cancer.

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## Table of Contents

**Clinical and Functional Analysis of p73R1 Mutations in Prostate Cancer**

**Wanguo Liu, Mayo Clinic, Rochester MN 55905**

## Introduction:

Prostate cancer is a complex genetic disease and its etiology very likely involves many genetic loci with no major gene with high penetrance. To identify such prostate cancer susceptibility genes, we have been using a novel approach based on mutation screening of candidate genes involved in the DNA damage-signaling pathway. Genomic instability is a common feature of all human cancers. The DNA damage-signaling pathway plays a critical role in maintaining genomic stability in response to DNA damage. The integrity of this pathway is essential for the prevention of neoplastic transformation, since several proteins involved in this pathway (such as p53, BRCA1, and ATM) are frequently mutated in human cancer. In a search for p73-dependent DNA damage-responsive genes, we isolated a p73-upregulated gene (*p73R1*) which is identical to the *p53AIP1* gene (ref. 1) that is activated by cisplatin-induced DNA damage. We have identified several deleterious germline mutations in this gene in approximately 3.2% (17/532) of primary or sporadic prostate cancer but only in 0.6% (2/331) of unaffected men (Fisher's exact test, $P = 0.016$). We, therefore, propose to study in detail the involvement of this gene in familial prostate cancer families which will be the first step towards providing evidence that *p73R1* is a prostate cancer susceptibility gene. The **objective** of this project is to identify the genetic role of p73R1 in prostate cancer development and to determine the functions and mechanisms of p73R1 in tumorigenesis. Our **hypothesis** is that *p73R1* is a candidate prostate cancer susceptibility gene. **Three Aims** for this proposal are: (1) To determine whether *p73R1* mutations co-segregate with prostate cancer phenotype in familial prostate cancer families; (2) to explore whether *p73R1* mutations are associated with any clinical and pathological characteristics in patients with prostate cancers; and (3) to determine the functional role of p73R1 in tumorigenesis. We have developed an accurate and sensitive Denaturing HPLC protocol to detect *p73R1* mutations. We will screen for *p73R1* mutations in 163 familial prostate cancer families and in 1,000 tumor samples collected at Mayo Clinic. Model-free genetic linkage analysis and statistical analysis will be performed to determine the co-segregation of *p73R1* mutations in prostate cancer families and any clinicopathological significance in patients with mutations and those without. *p73R1* mutants will be generated and expressed in mammalian cell systems to determine if the mutations fail to induce apoptosis and suppress cell growth.

**Body:** The tasks, which we proposed to fulfill in Year 1 and the accomplishments associated with each task, are summarized below:

<u>Specific Aim 1:</u> **To identify *p73R1* mutations and to study the segregation of these mutations in familial CaP:** We will screen for *p73R1* mutations in 163 families with familial CaP to determine whether the mutations segregate with the CaP phenotype.

In our preliminary study, we identified two truncating germline *p73R1* mutations (Ser32Stop and Arg21insG) in sporadic prostate cancer and in clinical CaP tissues. We showed that these mutations associated with prostate cancer risk by comparison between 400 men with sporadic CaP and 331 unaffected men (Fisher's exact test, P = 0.016), with an odds ratio of 5.5 (95% confidence interval 1.2-24.7) (Table.1). In addition, these two truncating mutations were not detected in any of the 403 non-

prostate tumor specimens including 127 breast cancer, 110 ovarian cancer, 72 gastric cancer, and 94 neuroblastomas (Table 1), suggesting that the *p73R1* truncating mutations are probably specific for prostate cancer. The familial, sporadic CaP cases, and the unaffected men controls used in this study are described in one of our previous publications (see ref. 2).

**Table 1. Frequencies of *p53AIP1* Mutations in Patients with Prostate Cancer, Non-prostate Cancers, and Unaffected Control Subjects**

| DNA samples | Cases | Ala7Val | Ser32Stop | Arg21insG | Arg102Glu | Pro105Ser |
|---|---|---|---|---|---|---|
| Clinic CaP tissues | 132 | 6 (4.5%) | 2 | 2 | 54 (40.9%) | 53 (40.2%) |
| Sporadic CaP blood | 403 | 7 (1.7%) | 3 | 10 | - | - |
| Unaffected men blood | 327 | 7 (2.1%) | 1 | 1 | - | - |
| Neuroblastoma tissues | 94 | 3 (3.2%) | 0 | 0 | - | - |
| Ovarian cancer tissues | 110 | 4 (3.6%) | 0 | 0 | - | - |
| Breast cancer tissues | 127 | 5 (3.9%) | 0 | 0 | - | - |
| Gastric cancer tissues | 72 | 1 (1.4%) | 0 | 0 | - | - |

In the last year, we completed the screening for *p73R1* mutations in all family members of 166 prostate cancer families collected at Mayo Clinic. A total of 596 individuals from these families, including 440 affected men, 55 unaffected men, and 101 females, were analyzed (Table 2). We detected 6 Arg21insG mutations in 440 affected men but not in any unaffected men or any females in these families. The frequency of the germline *p73R1* truncating mutations in familial prostate cancer (FPC) is 1.4% (6/440). Although the mutation frequency in FPC is higher than 0.6% (2/327) in population-based controls, it is much lower than 3.2% (13/403) in sporadic prostate cancer as we previously studied. In addition, the six Arg21insG mutations were detected in six unrelated families, indicating that the *p73R1* mutations are not likely associated with FPC.

**Table 2. Frequencies of *p53AIP1* Truncating Mutations in Familial Prostate Cancer**

| DNA samples | Family members | Ser32Stop | Arg21insG | Frequency |
|---|---|---|---|---|
| ***Mayo 166 FPC families*** | | | | |
| Affected men | 440 | 0 | 6 | 1.4% |
| Unaffected men | 55 | 0 | 0 | |
| ***Michigan 134 FPC families*** | | | | |
| Affected men | 308 | 2 | 6 | 2.6% |
| Unaffected men | 39 | 0 | 0 | |
| ***Johns Hopkins 144 FPC families*** | | | | |
| Affected men | 246 | 1 | 1 | 0.8% |
| Unaffected men | 6 | 0 | 0 | |

| | | | |
|---|---|---|---|
| Total affected men | 994 | 3 | 13 | 1.6% |
| Total unaffected men | 100 | 0 | 0 | 0.0% |

Since the average number of affected men in our FPC families is smaller (4.2 affected per family) than those collected at Johns Hopkins and the University of Michigan (over 5 affected per family), we collaborated with the two groups and analyzed two additional sets of FPC families from these two groups to rule out the possibility that *p73R1* mutations may only confer susceptibility to large FPC families. As shown in Table 2, we found eight *p73R1* frameshift (two Ser32Stop and six Arg21insG) mutations in 308 affected men in 134 Michigan FPC families; the frequency is 2.6%. However, we only detected 2 frameshift (one Ser32Stop and one Arg21insG) mutations in 246 affected men from 144 Johns Hopkins FPC families; the frequency is only 0.8% (Table 2).

So far, we have screened for *p73R1* truncating mutations in a total of 444 HPC families. We detected 16 truncating mutations in 994 (1.6%) of the affected men and no truncating mutations were detected among the 100 unaffected men from these families, (Table 2). Although the data reflects a similar trend as that observed for other comparison, the frequency is lower than that detected in the sporadic cases (3.2%). Other genes may play a more prominent role for prostate cancer susceptibility in the familial cases. Clearly, additional studies are needed to explain this difference.

**Specific Aim 2: To determine the clinicopathological significance of *p73R1* mutations in patients with sporadic CaP:** We plan to screen for *p73R1* mutations in an additional 1,000 unselected CaP tumor samples to determine possible associations with age, disease stage, PSA levels, aneuploidy, etc. Statistical analyses will be performed to assess the increased risk in patients with *p73R1* mutations compared to those without or to 332 unaffected men in the normal population.

Since there were no obvious genetic roles of the *p73R1* truncating mutations in FPC, we focused our effort on analysis of the clinicpathological significance of *p73R1* mutations in patients with sporadic CaP as we proposed in Aim 2. We have collected more than 1,000 freshly frozen prostate tumor tissues so far. These tumors are paired with normal tissues. As we proposed, we have H&E stained slides from each of the CaP. Dr. John Cheville has examined the slides and marked the tumor area on each slide. The TACMA core facility lead by Dr. Wilma Lingle has performed macrodissection on most of the tumors. Tissue slices from approximately 400 paired CaP tumor/normal have been prepared and DNAs have been isolated. We are ahead of the schedule of the proposal. We will finish the DNA isolation from 1,000 pairs of CaP by March 2005 and start mutational screening in these tumor specimens to determine the clinicopathological significance of the *p73R1* mutations in prostate cancer.

**Key Research Accomplishments:**
1) We finished screening for germline *p73R1* mutations in 994 affected men, 100 unaffected men, and 150 females from a total of 444 FPC families collected at three institutions (Mayo, Johns Hopkins, and University of Michigan). The *p73R1* frameshift mutations were found in 1.6% of the affected men but in none of the 100 unaffected men in these FPC families. Our data suggest that the frequency of the *p73R1* mutations in FPC (1.6%) is lower than those detected in sporadic cases (3.2%). Although the data reflects a similar trend observed for other comparisons, other genes may

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play a more prominent role for prostate cancer susceptibility in the familial cases. Clearly, additional studies are needed to explain this difference.

2) We have processed about 400 pairs of unselected clinical CaP tumor and their matched normal tissues. We performed macrodissection on all of the tumor tissues and isolated the DNA from the tissue slices for further analysis of the clinicopathological impacts of the p73R1 mutations in prostate cancer, which is described in Aim 2 of this proposal.

**Reportable outcomes:**
Germline *p73R1* mutations are implicated in 3% of men with sporadic prostate cancer.

**Conclusions:**
We have shown that germline *p73R1* truncating mutations increase risk for men to develop sporadic prostate cancer by 5-6 fold and further supports the concept that the mutant alleles in the DNA damage-response genes play an important role in the development of sporadic prostate cancer. However, analysis of the *p73R1* mutations in 444 FPC families indicate that the frequency of *p73R1* mutations in FPC (1.6%) is lower than that in sporadic cases (3.2%). Although the data reflects a similar trend observed for other comparisons. Other genes may play a more prominent role for prostate cancer susceptibility in the familial cases. Clearly, additional studies are needed to explain this difference.

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**Appendices:**
1. Xianshu Wang,[1] Ken Taniguchi,[1] Ratnam S. Seelan,[1] Liang Wang,[1] Shannon K McDonnell,[2] Chiping Qian,[1] Kaifeng Pan,[3] Youyong Lu,[3] Ping Yang,[2] Viji Shridhar,[1] Fergus J. Couch,[1] Donald J. Tindall,[4] Jennifer L. Beebe-Dimmer,[5] Kathleen A. Cooney,[5] William B. Isaacs,[6] Steve J. Jacobsen,[2] Daniel J. Schaid,[2] Stephen N. Thibodeau,[1] Wanguo Liu[1,7] Germline *p53AIP1* Mutations are Implicated in 3% of Men with Sporadic Prostate Cancer. **Am J Hum Genet** (submitted to AJHG and in communication).

# Germline *p53AIP1* Mutations are Implicated in 3% of Men with Sporadic Prostate Cancer[1]

Xianshu Wang,[1] Ken Taniguchi,[1] Ratnam S. Seelan,[1] Liang Wang,[1] Shannon K McDonnell,[2] Chiping Qian,[1] Kaifeng Pan,[3] Youyong Lu,[3] Ping Yang,[2] Viji Shridhar,[1] Fergus J. Couch,[1] Donald J. Tindall,[4] Jennifer L. Beebe-Dimmer,[5] Kathleen A. Cooney,[5] William B. Isaacs,[6] Steve J. Jacobsen,[2] Daniel J. Schaid,[2] Stephen N. Thibodeau,[1] and Wanguo Liu[1, 7]

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Running title: Germline *p53AIP1* mutations in sporadic prostate cancer risk

Key Words: *p53AIP1*, germline mutations, prostate cancer risk.

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## SUMMARY

The DNA damage-signaling pathway has been implicated in the development of prostate cancer. Germline mutations in several genes (*BRCA1, BRCA2, and CHEK2*) whose products are involved in this pathway have been associated with increased risk for this disease. To identify additional genes in this pathway that might confer susceptibility to prostate cancer, we analyzed a recently identified DNA damage-response gene, *P53AIP1* (a gene encoding for p53-regulated Apoptosis-Inducing Protein 1) for mutations in prostate cancer. Five novel mutations were identified. The mutations are germline, since they were present in matched normal tissues and blood samples. Importantly, the two truncating mutations (Ser32Stop and Arg21insG) were found in 3% (4/132) of unselected prostate tumor samples, whereas neither mutation was found in 95 clinic-based controls nor in 403 non-prostate tumor samples. Genotyping of an additional 403 men with sporadic prostate cancer showed a frequency of 3.2% (13/403) in contrast to 0.6% (2/327) in an additional 327 population-based controls (Fisher's exact test, $P = 0.016$), with an odds ratio of 5.4 (95% confidence interval $1.2 - 24.2$). Analyses of 994 affected men from 444 families showed a lower frequency of 1.6% suggesting a lesser involvement in familial prostate cancer. Loss of heterozygosity (LOH) analysis revealed that 2 of 6 tumors with *p53AIP1* mutations lost wild-type alleles, suggesting that p53AIP1 acts as a tumor suppressor. Overall, our data suggest that germline *p53AIP1* truncating mutations may predispose men to prostate cancer and further supports the concept that the mutant alleles in the DNA damage-response genes play an important role in the development of sporadic prostate cancer.

Many lines of evidence have shown that genetics plays a crucial role in the development of prostate cancer [MIM 300200](Gronberg 2003; Schaid 2004). In the last several years, a growing body of evidence suggests that a number of rare highly penetrant loci may contribute to Mendelian inheritance of prostate cancer and that other genetic alterations contributing to the majority of non-Mendelian inheritance of prostate cancer are likely to be the common low-penetrant alleles (Nwosu et al. 2001). These alleles could bear function-associated polymorphisms in the regulatory genes, such as the androgen receptor gene (AR [MIM 313700]), or mutations in genes associated with certain signaling pathways that are involved in prostate tumorigenesis (Mononene et al. 2000; Ikonen T et al. 2001).

The DNA damage-signaling pathway is essential for the prevention of genomic instability, a common feature of all human cancers including prostate cancer. Previous studies have shown that defects in the key components of this pathway (p53 [MIM 191170], BRCA1 [MIM 113705], and BRCA2 [MIM 600185]) are associated with prostate cancer risk (Gumerlock et al. 1997; Edwards et al. 2003). Moreover, the study of a genomic instability-based transgenic mouse model for prostate cancer demonstrates the presence of a similar phenotype in early stages of human prostate cancer and further suggests that the genomic instability could be an early event in this disease (Voelkel-Johnson et al. 2000). Recently we reported the involvement of germline mutations in *CHEK2* [MIM 604373], an up-stream regulator of p53, in prostate cancer and suggested an increased risk for men with *CHEK2*-mutations to develop prostate cancer (Dong et al. 2003). Thus, it is very likely that other genes that participated in the DNA damage-signaling pathway could be targets for mutations in prostate cancer tumorigenesis.

*P53AIP1* [MIM 605426] is a downstream target of p53 and is induced by DNA damage (Oda et al. 2000). This gene generates three transcripts ($\alpha$, $\beta$, and $\gamma$) by alternative splicing. The $\alpha$ and $\beta$ forms of p53AIP1 are localized to the mitochondria and induce apoptosis through dissipation of mitochondria membrane potential (Oda et al. 2000). The expression of *p53AIP1* and p53AIP1-induced apoptosis are closely correlated with phosphorylation of p53 at Ser-46, indicating that p53AIP1 plays an important role in mediating p53-dependent apoptosis. Moreover, p53AIP1 interacts with bcl-2, an inhibitor of apoptosis, in mitochondria (Matsuda et al. 2002). This event induces the release of cytochrome c from mitochondria and very likely regulates p53AIP1-mediated apoptosis through regulation of the mitochondrial membrane potential. P73 [MIM 601990], the homolog of p53, also induces transcription of the proapoptotic gene *p53AIP1* after being acetylated by p300 (Costanzo et al. 2002). These studies suggest that p53AIP1 is crucial for regulation of both p53- and p73-dependent DNA damage-signaling pathways and disruption of the function of p53AIP1 in p53- and p73-mediated apoptosis could play a role in cancer development. Since p73 is not mutated in prostate cancer (data not shown) and the mutation frequency in p53 is low (3-30%) in prostate cancer but much higher in other cancers, *p53AIP1* could be an ideal mutation target for prostate cancer susceptibility.

To determine whether disruption of the function of p53AIP1 in the p53- or p73-dependent apoptotic pathway is associated with prostate cancer risk, we first screened

*p53AIP1α* for mutations in 132 primary prostate tumor specimens from an unselected series of

samples collected at Mayo Clinic between 1997-1998. The entire coding and exon/intron junction

sequences of this gene were amplified by PCR using three primer pairs *p53AIP1*e2F/R (5'-

AAATGAGGAGAAGCCAAGTT3' and 5'CGGCACCACGGTGAGA-3'), *p53AIP1*e3F/R

(5'-AACCATCCAAGAGACGG3' and 5'ATCACTTAATTCTATCACGG-3'), and

*p53AIP1*e4F/R (5'-AAGGACTCCATACGTTTTGC3' and 5'GCTGGAGCCATTTCTCGAC-

3'). The PCR products were screened for sequence mismatches by denaturing high-performance

liquid chromatography (DHPLC) followed by direct sequence analysis (Liu et al. 1998). Three

unique non-synonymous sequence variations (C20T, Ala7Val; A304G, Arg102Glu; C313T,

Pro105Ser), one nonsense mutation (C95A, Ser32Stop), and one frameshift mutation due to a one

base-pair insertion (64insG, Arg21insG)(Figure 1) were identified. The two *p53AIP1* truncating

mutations (Ser32Stop and Arg21insG) were present in 3% (4/132) of the tumor samples (Table

1). The three missense mutations were present in 4.5-41% of prostate tumor specimens (Table

1). All of the 5 mutations identified in *p53AIP1* were present in both tumor specimens and

matched normal tissues, indicating that they were germline in nature (data not shown). We then

tested for the presence of these mutations within the normal population by screening for

*p53AIP1* mutations in 95 normal individuals. These individuals, which included 38 females and 57

males were seen at the Mayo Clinic during 1998-1999 as part of a clinical evaluation. There was

no history of cancer for any of these individuals at the time of blood collection and none of the

men were tested for elevated levels of PSA. Except for the three missense mutations that were

detected at slightly lower frequencies than those detected in prostate tumor samples (Table 1),

we did not detect the two truncating mutations in any of the normal individuals. These data

suggested that the two *p53AIP1* truncating mutations are associated with prostate cancer risk.

To further evaluate the significance of the two *p53AIP1* truncating mutations in the

predisposition to sporadic prostate cancer, we assessed their frequencies in an additional 403

men with sporadic prostate cancer and in an additional 327 population-based controls (all male).

These two sets of samples have been previously published in other gene association studies

except that 4 controls were dropped due to the occurrence of prostate cancer and 3 sporadic

cases were added (Dong et al. 2003; Wang et al. 2003). The patients with sporadic prostate

cancer were collected at the Mayo Clinic and were selected from respondents to a family history

survey that reported no family history of prostate cancer (Schaid et al. 1998; Wang et al. 2002).

The diagnosis of prostate cancer was confirmed by pathology reports. Prostate specific antigen

(PSA) values at diagnosis were available for 326 men, with a median value of 7.2 and with 76%

having values of 4 or greater. The median age for this group was 65.0 (range 46.0 – 79.0). The

unaffected control subjects were recruited from a sampling frame of the local population provided

by the Rochester Epidemiology Project (Melton 1996), 475 men were randomly selected for a

clinical urologic examination (Oesterling et al. 1993). This clinical examination included digital

rectal examination (DRE) and transrectal ultrasound (TRUS) of the prostate, abdominal

ultrasound for post-void residual urine volume, measurement of serum levels of prostate-specific

antigen (PSA) and creatinine, focused urologic physical examination, and cryopreservation of

serum for subsequent sex hormone assays. Any patient with an abnormal DRE, elevated serum

PSA level, or suspicious lesion on TRUS was evaluated for prostatic malignancy. If the DRE and

TRUS were unremarkable but the serum PSA level was elevated (>4.0 ng/ml), then a sextant

biopsy (three cores from each side) of the prostate was performed. An abnormal DRE or TRUS

results, regardless of the serum PSA level, prompted a biopsy of the area in question. In addition,

a sextant biopsy of the remaining prostate was performed. Those men who were found to be

without prostate cancer on the basis of this extensive workup at baseline or at any of the follow-

up examinations through 1994 were used for the control population. Three hundred and twenty-

seven of these individuals participated in this particular study. The median age of these men was

59.6 (range 49.0 – 89.0). All of the participants in this study gave full informed consent and were

approved by Mayo Institutional Review Board. Analysis of *p53AIP1* mutations in these two

groups indicated thatthe Ser32Stop and Arg21insG mutations were present in 13 of 403 (3.2%)

men with sporadic prostate cancer and in 2 of 327 (0.6%) unaffected men (Table 1), a

statistically significant difference (Fisher's exact test, $P = 0.016$). The odds ratio was 5.4, with a

95% confidence interval of 1.2 – 24.2. These results suggest that men carrying the germline

*p53AIP1* truncating mutations have an increased risk for developing prostate cancer by 5-6 fold.


We also examined the *p53AIP1* mutations in other types of tumors. Genotyping of the

two truncating mutations and the C20T (Ala7Val) missense mutation was performed in 403

tumor samples including 127 breast cancer, 110 ovarian cancer, 72 gastric cancer and 94

neuroblastomas (Table 1). Although the C20T missense mutation identified in prostate cancer

was also present in these cancers with a similar frequency, we did not detect *p53AIP1* truncating

mutations in any of these non-prostate tumor samples, suggesting that the *p53AIP1* truncating

mutations are probably specific for prostate cancer. However, these results further support the

finding of a low frequency of the 2 mutations in the population. Additional studies will be needed to analyze additional tumor types and more samples for each tumor type in order to determine whether the *p53AIP1* truncating mutations are unique to prostate cancer.

The results from this study and the studies of *BRCA1, BRCA2, CHEK2,* and *NBS1* [MIM 602667] in prostate cancer suggest that germline mutations in these genes involved in DNA damage-signaling pathway contribute to genetic susceptibility of prostate cancer (Dong et al. 2003; Cybulski et al. 2004). Although the majority of the mutations for these genes were found in sporadic prostate cancer, some of the mutations were observed in both familial and sporadic prostate cancers (Kirchhoff et al. 2004). To explore the possibility that the two truncating mutations of *p53AIP1* might also play a role in hereditary prostate cancer, we further analyzed a total of 994 affected men and 100 unaffected men from 444 families with prostate cancer. This included 166 families collected at Mayo Clinic, 144 at Johns Hopkins, and 134 at University of Michigan (Wang et al. 2003). Although no truncating mutations were detected among the 100 unaffected men from these families, we detected 16 truncating mutations in 994 (1.6%) of the affected men (Table 2). Although the data reflects a similar trend as that observed for other comparison, the frequency is lower than that detected in the sporadic cases (3.2%). Other genes may play a more prominent role for prostate cancer susceptibility in the familial cases. Clearly, additional studies are needed to explain this difference.

Since most of the genes participating in the DNA damage-signaling pathway and conferring susceptibility to prostate cancer are tumor suppressor genes, we performed loss of

heterozygosity (LOH) analysis on 6 available tumors harboring *p53AIP1* truncating mutations and their matched normal adjacent tissues. Analysis of the DNA isolated from Laser Capture Microdissected tumor tissues by direct sequence revealed that loss of the wild-type alleles were present in two of the samples suggesting that *p53AIP1* is a potential tumor suppressor gene (data not shown). However, the mechanism by which *p53AIP1* truncating mutations contribute to prostate cancer risk is still not well defined. Recently, Matsuda et al., demonstrated that p53AIP1 is localized to the mitochondria and binds to bcl-2 thereby participating in the apoptosis pathway (Matsuda et al. 2002). Whether the *p53AIP1* mutants are able to localize to mitochondria or affect the dissipation of mitochondria membrane potential leading to apoptosis remains to be elucidated.

In conclusion, our data provide the first evidence that germline mutations in *p53AIP1* may be associated with prostate cancer risk. Although we estimate the risk to be large, with an odds ratio of 5.4, the rarity of the mutations translates to a wide confidence interval in this risk, 1.2 – 24.2. Larger studies are required to refine this risk estimate. These findings together with previous findings that germline mutations in four other DNA damage-responding genes (*CHEK2, BRCA1, BRCA2,* and *NBS1*) also confer prostate cancer susceptibility in the general population, further highlights the importance of the integrity of the DNA damage-signaling pathway in prostate cancer development. Although the mechanism by which germline mutations in *p53AIP1* lead to prostate cancinogenesis remains to be elucidated, these mutations in prostate cancer may facilitate early diagnosis of prostate cancer and provide additional insights into the biology of this malignancy. None-the-less, independent studies will be required to further

support these findings due to the complexity of the disease. Also, additional research is needed to

determine how the mutant p53AIP1 induces prostate tumorigenesis and whether other genes in

the same DNA-damage-signaling pathway also confer susceptibility to prostate cancer.

## ACKNOWLEDGMENTS

## ELECTRONIC-DATABASE INFORMATION

The accession numbers and URLs for data presented herein are as follows:

Genbank, http://www.ncbi.nih.gov/Genbank/ (for *p53AIP1* [accession number AB045830])

Online Mendelian Inheritance in Man (OMIN),

http://www.ncbi.nih.gov/entrez/query.fcgi?db=OMIM (for prostate cancer [MIM 300200], AR

[MIM 313700], p53 [MIM 191170], BRCA1 [MIM 113705], BRCA2 [MIM 600185],

CHEK2 [MIM 604373], P53AIP1 [MIM 605426], P73 [MIM 601990], and NBS1 [MIM

602667]).

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prostate cancer risk. Nat Genet 35:128-9.

**Table 1. Frequencies of *p53AIP1* Mutations in Patients with Prostate Cancer, Non-prostate Cancers, and Unaffected Control Subjects**

| DNA samples | Cases | Ala7Val | Ser32Stop | Arg21insG | Arg102Glu | Pro105Ser |
|---|---|---|---|---|---|---|
| Clinic CaP tissues | 132 | 6 (4.5%) | 2 | 2 | 54 (40.9%) | 53 (40.2%) |
| Sporadic CaP blood | 403 | 7 (1.7%) | 3 | 10 | - | - |
| Normal control blood | 95 | 3 (3.2%) | 0 | 0 | 28 (29.4%) | 28 (29.4%) |
| Unaffected men blood | 327 | 7 (2.1%) | 1 | 1 | - | - |
| Neuroblastoma tissues | 94 | 3 (3.2%) | 0 | 0 | - | - |
| Ovarian cancer tissues | 110 | 4 (3.6%) | 0 | 0 | - | - |
| Breast cancer tissues | 127 | 5 (3.9%) | 0 | 0 | - | - |
| Gastric cancer tissues | 72 | 1 (1.4%) | 0 | 0 | - | - |

**Table 2. Frequencies of *p53AIP1* Truncating Mutations
in Familial Prostate Cancer**

| DNA samples | Family members | Ser32Stop | Arg21insG | Frequency |
|---|---|---|---|---|
| Mayo 166 FPC families | | | | |
| Affected men | 440 | 0 | 6 | 1.4% |
| Unaffected men | 55 | 0 | 0 | |
| | | | | |
| Michigan 134 FPC families | | | | |
| Affected men | 308 | 2 | 6 | 2.6% |
| Unaffected men | 39 | 0 | 0 | |
| | | | | |
| Johns Hopkins 144 FPC families | | | | |
| Affected men | 246 | 1 | 1 | 0.8% |
| Unaffected men | 6 | 0 | 0 | |
| | | | | |
| Total affected men | 994 | 3 | 13 | 1.6% |
| Total unaffected men | 100 | 0 | 0 | 0.0% |

**Figure legends:**

**Fig. 1.** DHPLC and sequence analyses reveal *p53AIP1* mutations in human prostate tumors. A.

DHPLC analysis shows the normal (green) and abnormal (red) DHPLC profiles for tumor

samples with five *p53AIP1* mutations. B. Sequence analysis demonstrates the five *p53AIP1*

mutations. Upper panels depict the regions from wild-type alleles and lower panels show the

corresponding mutant alleles in heterozygous conditions. The arrows mark the location of each

mutation.